Biodegradation of Oil and Corexit 9500A by Arctic Marine Microorganisms

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Oil Seeps in Alaska

NRC, 2003
Data gaps remain regarding the taxonomic identity of Arctic oil-degraders and rates of oil biodegradation in seawater.

- What has been reported for seawater relies primarily on culture-based methods that may create results unrepresentative of the sampled environment.
Chemical Dispersants (Corexit 9500A)

– Dispersants increase bioavailability of oil by increasing surface area. (Beal and Betts, 2000; Rosenberg, 1993)

– Smaller oil droplets biodegrade faster than larger droplets. (10 µM vs. 30 µM, Brakstad et al., 2015)

– Dispersed oil biodegrades faster than a surface oil slick. (Prince & Butler, 2014)
  • Adds more chemicals to the environment

Image courtesy of ITOPF

Chemical dispersants can suppress the activity of natural oil-degrading microorganisms

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Corexit Biodegradation Studies
Currently no published reports exist for the primary biodegradation of Corexit in Arctic seawater.

Metabolic functions of these deep-sea microbial communities may not be representative of Arctic communities.

The rates at which Corexit and oil biodegrade in Arctic environments are still unknown (NRC, 2014).
Corexit Biodegradation Studies

It is likely that microorganisms are capable of biodegrading both oil and dispersant components

During the Deepwater Horizon oil spill

• Known oil-degrading taxa (e.g. *Colwellia*) were enriched by chemically dispersed oil in a deep-water plume (Redmond and Valentine, 2012; Dubinsky et al., 2013)

In Gulf of Mexico seawater incubations

• Similar species (e.g. *Colwellia*) also increased in abundance in response to:
  – Corexit-only (Kleindienst et al., 2015)
  – Chemically dispersed oil (Baelum et al., 2012; Chakraborty et al., 2012)
What’s in Corexit 9500A?

**Surfactant components**
- Anionic surfactant
  - 18% (w/w) dioctylsulfosuccinate (DOSS)
- Non-ionic surfactants
  - 18% (w/w) Tween 80
  - 4.6% (w/w) Tween 85
  - 4.4% (w/w) Span 80

**Other components**
- Dipropylene glycol butyl ether
- 2-butoxy-ethanol
- Petroleum distillates
- And others

Place et al., 2016, Parker 2014, U.S. EPA
Corexit Contains Hydrocarbons

Hydrocarbons are present in

- the solvent fraction of Corexit
  – made of petroleum distillates
    • include alkanes (C₉ - C₁₆) and paraffins
  
- the side chains of DOSS (Seidel et al., 2016)

Bacteria may use similar genetic pathways when biodegrading oil and Corexit compounds.
Biotransformation of Oil

The microorganisms that increase in abundance in an oiled environment are most likely the oil-degraders.
Biodegradation Genes are Passed Between Bacterial Cells

It is highly likely that genes involved in the biodegradation of Corexit are also passed from cell to cell.

The presence or absence of specific degraders should not be used a proxy for biodegradation efficiency or inhibition.
Rates of Biodegradation

• Biodegradation rates are thought to be slower in Arctic than temperate regions

• Some microorganisms are adapted to low temperatures (Feller, 2003)
  – Similar oil biodegradation rates in cold and temperate environments have been reported (Braddock and McCarthy, 1996; Margesin and Schinner, 1997; Gibb et al., 2001)
Rates of Biodegradation

Oil properties at low temperature more likely limit oil biodegradation than metabolism (Bagi et al., 2013; Deppe et al., 2005)

• Temperature increases evaporation and diffusion (Honrath & Mihelcic, 1999)
  – Which can result in more oil lost in temperate vs. Arctic environments (Prince et al., 2012 vs. McFarlin et al., 2014)
Biodegradation of Oil & Corexit 9500A in Arctic seawater (Chukchi Sea, Alaska)

Objectives

• Quantify the chemical loss of ANS crude oil and surfactant components of Corexit 9500A
  – Calculate biodegradation rates (assist fate models)

• Identify bacteria and genes that may be involved in oil and Corexit biodegradation
  – Determine similarities between Arctic and Gulf of Mexico microbial communities
Surface Seawater Collection Incubation Studies

Two water collections
- September 2013
- October 2013
- ~ 90 km from shore
- Open water season

Water collected by Chukchi Sea Environmental Studies Program
Biodegradation Incubations
Chemical Loss

Seawater Incubations (2 °C)

1. Seawater (800 mL)
2. Nutrient Medium (16 mg/L)
3. ANS Crude Oil (15 mg/L)
   OR
   Corexit 9500A (15 mg/L)

Time points: 5, 10, & 28 days
• Open to the atmosphere
• Constantly stirred
Oil Analysis Methods

Primary Biodegradation

• Total measurable hydrocarbons (C_8-C_40) were extracted and analyzed with GC-MS.
• Values were normalized to 17α(H),21β(H)-hopane as a conserved internal marker within the oil.
• Biodegradation was determined as % loss from time zero incubations.
• Abiotic losses were determined in sterile controls and subtracted
% Biodegradation of Oil

Table 1. Mean Percent Loss of Oil at 2°C. Letters correspond to significant differences among time points \((p < 0.05; \text{ MRPP}; n = 3)\). Error bars represent standard deviation.
Corexit Analysis Methods

*Incubations contained Corexit-Only*

- Surfactant compounds were extracted and analyzed with LC-MS/MS.
  - DOSS
  - Non-ionic surfactants

- Biodegradation was determined as % loss from time zero incubations.
Biodegradation of Corexit 9500A

Figure 1. Mean concentration of DOSS at 2 °C. Incubations contained Arctic seawater (800 mL), Corexit (15 mg/L), and nutrients (16 mg/L; Bushnell Haas ) (n = 3).
Non-ionic Surfactants Below Detection Limits at Day 28

Table 2. Mean concentration of non-ionic surfactant components of Corexit at 2°C. Incubations contained Arctic seawater (800 mL), Corexit (15 mg/L), and nutrients (16 mg/L Bushnell Haas). Letters correspond to significant differences among time points \( (p < 0.05; \text{MRPP}; n = 3) \).

Sterile control

detection limits: 5.5 µg/L, 15 µg/L, and 6.5 µg/L, respectively
Microbial Community Response
to Oil and Corexit 9500A
Microbial Community Analysis

Objective

• Determine which microbes and genes increased in abundance in response to
  – Oil (15 mg/L)
  – Corexit 9500A (15 mg/L)
*Indicates likely degraders

Methods

• Sequencing 16S rRNA genes (V6), Illumina MiSeq
  Determines presence and relative abundance of bacteria

• GeoChip Microarray 5.0
  Determines presence and abundance of oil-degrading genes
Microbial Community Structure

Figure 2. Relative Abundance of Most Abundant Genera at 2°C. Day 0 and day 28 time points are shown for incubations containing no added carbon, oil (15 mg/L), and Corexit (15 mg/L).
Response of Colwelliaceae Family to Oil or Corexit

Figure 3. Relative abundance of sequences classified in the Colwelliaceae family. Day 0, 10, and 28 time points are shown for seawater incubations containing no added carbon (N), oil (15 mg/L), or Corexit (C; 15 mg/L) at 2°C.
Figure 4. Relative abundance of sequences classified in the Rhodobacteraceae family to oil or Corexit. Day 0 and 28 time points are shown for incubations with no added carbon (N), oil (15 mg/L), or Corexit (C; 15 mg/L) at 2°C.
Alkane monooxygenase (alkB) genes increased in abundance in response to oil and Corexit 9500A.

Figure 5. Relative Abundance of alkB Genes. Day 0 and day 28 time points are shown for October 2013 incubations containing no oil (NO), with oil (15 ppm) and with Corexit 9500A (COR; 15 ppm) at 2°C.
Arctic Oil biodegradation in Surface Seawater

What we know

• Similar extents of biodegradation of whole ANS crude oil (2.5-15 mg/L) can occur in near-shore (McFarlin et al., 2014) and offshore (this study) Arctic seawater (-1 °C to 2 °C).
  – 30-36% of unweathered ANS crude oil biodegraded within 28 days

• The extent of oil biodegradation in Arctic seawater (2 °C) is lower than temperate seawater (8 °C; Prince et al., 2013) within 28 days
  – 36% (Arctic) vs. 69% (Temperate)

• Rates of oil biodegradation in Arctic seawater are comparable to rates in temperate seawater
  – 0.011 gC/m³*d (18 °C, temperate seawater, Atlas & Bartha, 1973)
  – 0.015 gC/m³*d (1 °C, sub-Arctic, Laake et al., 1984)
Corexit Biodegradation in Arctic Seawater

• Arctic bacteria biodegraded a substantial amount of DOSS within 28 days at 2°C.
  – This is in contrast to DOSS biodegradation extents reported with deep-seawater from Gulf of Mexico
  • Kleindienst et al. (2015) reported an 8% loss of DOSS over 28 days (8°C).

• Non-ionic surfactants (Span 80, Tween 80, and Tween 85) were ~100% biodegraded (i.e. below detection limits) in Arctic seawater within 28 days at 2°C.
  – This is in agreement with Kleindienst et al. (2015)
Corexit Biodegradation in Arctic Seawater

• Corexit did not inhibit known oil-degrading taxa in Arctic seawater

• Taxa known to include oil-degrading bacteria (i.e. *Colwellia*) and functional genes known for oil-biodegradation (i.e. *alkB*) increased in response to both oil and Corexit

  – suggests that some oil-degrading bacteria may have the potential to biodegrade components in Coreexit
Conclusions

• Taxa known to include oil-degrading microorganisms are located throughout the Arctic water column (McFarlin et al., submitted) and within Arctic sea ice (Garneau et al., 2016).

• Oil degrading genes are also located throughout the Arctic water column (McFarlin et al., submitted) – gene presence doesn’t equate to expression

• These results support prior research indicating that substantial oil and Corexit biodegradation can occur in the marine environment without adding large amounts of nutrients or solutions containing oil-degrading microorganisms.
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Questions?